

Effect of Repetitive Transcranial Magnetic Stimulation on the Structural and Functional  
Connectome in Patients with Major Depressive Disorder

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### ABSTRACT

Through this whole-brain exploratory analysis, our aim is to study the effect of repetitive transcranial magnetic stimulation (rTMS) on the structural and functional connectivity of patients with major depressive disorder. Twenty-five currently depressed patients (age 21–68) participated in the study. Patients received daily 10-Hz rTMS over the left dlPFC five days/week for five weeks. Treatment response was assessed using the 24-item Hamilton Rating Scale for Depression (HAM-D-24) at baseline and after the course of TMS. MRIs were acquired within seven days prior to starting rTMS and within three days after the end of treatment. Using diffusion tensor images and resting-state fMRI data we computed the whole-brain functional and structural connectomes. We used graph theory techniques to characterize brain architecture to identify potential biomarkers for depression severity and response to treatment. The frontal pole, part of the midline core in the default mode network (DMN) and the exteroception compartment of the depression network (DN), was identified as a potential biomarker for depression severity. The intracalcarine cortex and lateral occipital cortex, neither part of the default mode network and depression network, were defined as potential biomarkers for treatment response. The subcallosal cortex, orbitofrontal cortex, and supramarginal gyrus were identified as potential biomarkers for treatment response and their change across the treatment protocol could explain the simultaneous effect of rTMS on structural and functional connectivity. Ultimately, the goal is to articulate specific hypotheses that will inform treatment strategies for patients with major depressive disorder.

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## INTRODUCTION

“All beauty comes from beautiful blood and a beautiful brain.”

— Walt Whitman, *Leaves of Grass*

The primary aim of this study was to use a method that makes it possible to investigate the structural and functional connectivity of the whole brain in patients with major depressive disorder, to assess the change in their connectivity over the course of repetitive transcranial magnetic stimulation treatment.

### **Major Depressive Disorder**

Major depression carries the heaviest burden of disability among mental and behavioral disorders (WHO; 2010). In 2014, an estimated 6.7% of all U.S. adults had at least one major depressive episode in the past year (NSDUH; 2015). Treatment resistant depression is hard to define, because treatment response is perceived differently for doctors and patients, for patients it is recovery and for doctors remission (Malhi, 2016). It is important that doctors and patients share expectations on the probability of remission to retain patients and avoid discouragement. A minimum of 8 weeks of treatment should be considered before making a change to accurately determine efficacy (Warden; 2007). In this study, we define treatment resistant depression as failure to respond to at least two previous antidepressant trials at adequate doses for 8 weeks during the current episode. The patients included in this sample were all classified as treatment resistant.

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According to the latest Diagnostic and Statistical Manual of Mental Disorders, to be diagnosed with depression a person must exhibit anhedonia, diminished interest or loss of pleasure, or depressed mood (American Psychiatric Association; 2013). In addition, this person must have at least four of the following symptoms for a 2-week period: significant weight change or appetite disturbance, sleep disturbance, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness, diminished ability to think or concentrate, and recurrent thoughts of death or suicidal ideation. Currently, there is no physical examination for depression, the diagnosis is based on the history and mental status evaluation to determine if the person has five of the symptoms. This examination must also rule out organic causes that imitate depression, such as infection, medication, tumor, an endocrine or neurologic disorder (American Psychiatric Association; 2013). As further research is conducted and potential biomarkers for depression severity are identified, it will be important to note organic causes that could affect these biomarkers.

### **Transcranial Magnetic Stimulation**

Transcranial magnetic stimulation (TMS) is a non-invasive tool that delivers rapidly pulsed magnetic field to the cerebral cortex to modulate the activity of neurons from a specific region. Repetitive TMS (rTMS) involves a repetitive train of pulses. A rapidly changing electrical current is passed from a coil placed on the scalp creating a time-varying magnetic field, this induces an electrical field in the cortex. The electrical field changes neuronal activity in the specific region being stimulated as well as the neuronal network to which it belongs (Levkovitz; 2015). rTMS can be administered at low frequencies which suppresses underlying cortical activity or high-frequency

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stimulation, as in this study, that results in an excitatory change, which is depolarization of the neurons in the brain region stimulated and subsequently the following regions in that pathway.

Repetitive transcranial magnetic stimulation (rTMS) of the left dorsolateral prefrontal cortex (dlPFC) is an FDA-approved treatment for medication-resistant major depressive disorder (FDA; 2011). Based on evidence and level of confidence the Agency for Healthcare Research and Quality (AHRQ) concluded that this protocol compared to sham treatment has: a greater decrease in depression severity, three times likelihood of response, six times likelihood of remission, and a greater improvement in health status and daily functioning (Gaynes; 2011).

The abnormality in the subgenual cingulate has been shown in various studies of depression; however, TMS has a limited capacity of stimulation for deeper limbic regions. The focus of TMS treatment has been on the left dlPFC since it is an accessible node of the pathway that has been defined as the depression network (Fox; 2012). An obstacle is determining the appropriate stimulation target within the dlPFC because it is a large area. Thus, future studies should use neuro-navigational procedures to accurately address which part is critically involved in depression (Fitzgerald; 2009). Focal brain stimulation can be improved by targeting based on connectivity. A structural MRI-based neuro-navigational procedure can help us accurately target different areas in the dlPFC and study which part is highly correlated with symptom improvement. Another obstacle is the heterogeneity of symptoms seen in patients with major depressive disorder. It is important to study various critical nodes that can improve different depression symptoms. As future research studies are conducted, we will be able to better understand the pathophysiology of depression and determine if this is a proper categorical representation of all the symptoms currently ascribed to major depressive disorder.

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### **Structural Connectome**

The term “connectome” is used to describe a comprehensive map of connections between the elements in the human brain. Diffusion tensor imaging (DTI) allows us to conduct a comprehensive analysis of the anatomical connections of the whole-brain by mapping the white matter tracts that link regions in the brain.

A brain network is the mathematical representation of the real-world complex system (Sporns; 2010). The network is defined by a collection of nodes (vertices), which represent brain regions from the parcellation of a specific atlas, and links (edges) between pairs of nodes. These links can represent anatomical, functional, or effective connections. To measure the organization of the brain’s anatomical architecture, graph theory metrics that quantify the connectivity profile of each node in relation to the network are used. These network metrics help us understand the importance of different brain regions, their local anatomical circuitry, and how they are embedded in the network.

Some potential sources of error when using this approach arise from the parcellation. An atlas defines the standard boundaries of each brain region; this template is then applied to individual brains. When parcellating it is possible to place a boundary in the middle of a brain region, due to individual differences that are not adjusted when that brain is fit into the template. Thus, some streamlines connecting brain areas can be lost if the region is defined incorrectly. This could result in false negatives when using graph theory metrics to quantify the connectivity of each region. Another source of error can be the fractional anisotropy threshold, if this is set too low then streamlines could be generated where they don’t exist. This would result in false positive connections between regions.

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In this study analysis of Diffusion Tensor Imaging data is performed to create a structural brain connectome for depressed patients pre and post rTMS treatment, and to assess the impact of rTMS treatment on brain connectivity.

### **Functional Connectome**

The term “functional connectome” is used to describe a comprehensive map of the functional connections between regions of the human brain (Biswal; 2009). Functional connectivity is a temporal correlation in activity between brain regions that may be anatomically unconnected, these correlations can be mapped into complex neural systems that make up a person’s functional connectome.

Resting-state functional MRI can be used to conduct a whole-brain analysis and examine multiple functional circuits without an *a priori* hypothesis. This was the approach of this study. Imaging the brain with fMRI at rest shows large amplitude spontaneous low-frequency fluctuations of neuronal activity that are correlated across functionally related areas. Reproducing this approach in many subjects proves a standard functional architecture; this allows us to compare inter-individual differences in connectivity patterns to further study biomarkers of development and the pathophysiology of brain disorders (Biswal; 2009). This technique holds promise as a clinical diagnostic tool because it has a simple experimental setting (Borchardt; 2016). In contrast, a task-based fMRI approach requires an *a priori* hypothesis. Limitations to this approach are that areas that are not necessarily involved in the same activity or pathway can still appear to be correlated if they showed activity during the scan. This spontaneous activity can be due to various causes and results in false positives.

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The aim of this study is to articulate specific hypotheses that will guide future study on the structural-functional connectivity of people with major depressive disorder.

### **Structural-Functional Connectivity**

Structural connectivity does not necessitate a functional connection, nor does a functional connection imply an anatomical connection. However, these two properties are interrelated and can be affected if the other is disrupted. Brain function is affected by changes in its structural substrate in response to environmental disturbances or disease, thus it is important to understand the relationship between these anatomical regions (Sporns; 2005). Structure and function are not completely mapped unto each other; functional connectivity can be derived from monosynaptic or polysynaptic anatomical circuits (Biswal; 2009). Also, certain factors such as arousal and sleep, can affect functional connectivity without altering structural connectivity. These factors must be taken into consideration to understand the relationship between structural and functional connectivity.

To transition from structure to function it is necessary to comprehend how cognition emerges from the connections between brain regions. The first step is to establish the structural network, its elements and connections, that can support a wide range of function and at the same time places a constraint on brain dynamics (Sporns; 2005). The anatomical connectivity, white-matter fiber tracts, integrates information from functionally segregated brain regions providing a framework for functional brain networks. Then methods that record brain activity can study how this fits into the structural connectivity. In this study, we used resting-state fMRI to record brain



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activity and compare the functional connectivity with the structural connectome of patients with major depressive disorder.

### **Brain Networks**

#### *Default Mode Network*

The default mode network is an anatomically defined brain system that participates in internal modes of cognition. It is activated when a person is not involved in an explicit task or engaged in another external interaction, and is instead in wakeful rest. This network has been associated with different functions: internal meditation, thinking about others, self-referential and affective decision making, remembering the past and thinking about the future. It consists of regions across the prefrontal cortex, lateral parietal cortex, medial temporal lobe, and across the anterior and posterior midline (Buckner et al.; 2008). Specifically, it has two subsystems that converge on a midline core (Buckner et al.; 2010). The Medial Temporal Lobe (MTL) subsystem is comprised of the ventral medial prefrontal cortex, posterior inferior parietal lobule, retrosplenial cortex, parahippocampal cortex, and hippocampal formation. The Dorsal Medial Prefrontal Cortex (dMPFC) subsystem includes the dorsal medial prefrontal cortex, temporoparietal junction, lateral temporal cortex, and temporal pole. They both converge at the midline core, that is comprised of the “hubs”: anterior medial prefrontal cortex and posterior cingulate cortex. The MTL subsystem is associated predominantly with a constructive function, specifically of recalling the past by constructing a mental scene based on memory and imagining the future through mnemonic imagery-based processes. In contrast, the dMPFC subsystem was mainly acting when considering the present. This includes affective self-reference and inferring mental states of other people. Both

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regions in the midline core exhibited functions from both subsystems. Personal significance, introspection about one's own mental states, and evoked emotion are associated with activity in the midline core (Buckner et al.; 2010). Some studies have found a link between the default mode network and the disordered self-referential thought of depression, suggesting this is a potential pathway for study to understand depression pathophysiology. They identified a stimulus-induced heightened activity and a failure to normally down-regulate activity in this pathway (Sheline et al.; 2009).

### *Depression Network*

The clinical symptom heterogeneity of major depressive disorder seen in patients poses a challenge to the definition of a specific brain circuit for this disorder. The aim is to define a depression network that can be the basis for optimal treatment selection for every patient. To achieve this, it is important to characterize the effect of different treatment interventions on the network. In this study, we aim to explore the effect of repetitive Transcranial Brain Stimulation on the brain networks.

To account for the variability between patients and the abnormal patterns of brain activation, a systems perspective is most suitable. A trigger on the mood circuit will lead to an array of symptoms and a diagnosis of depression, then the brain will exhibit an intrinsic adaptive or maladaptive response such as: over-correction, partial correction, under-correction, failed or absent response. The compensatory mechanism the person employs causes the heterogeneity in the symptoms presented. This model theoretically encompasses reported variability in research studies, heterogeneity of clinical symptoms, genetic risk factors, and the conceptual models of sustained stability in response to stress (Mayberg; 2009). The compensatory response can provide

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an insight into the best treatment for that person. A partial, sustained response could respond equally well to either pharmacological or psychological treatments. The extreme responses, overactive or underactive, require specific treatments such as cognitive behavioral therapy or interpersonal psychotherapy when overactive and medication augmentation or electroconvulsive therapy, vagus nerve stimulation, or repetitive transcranial magnetic stimulation when underactive (Rush; 2006). In the case of failure or an absent response, patients will require more aggressive interventions like deep brain stimulation.

Mayberg (2009) established a consensus on the circuit model of major depressive disorder, regions were grouped into four main compartments that reflect the behavioral dimensions of this disorder: exteroception, mood monitoring, mood regulation, and interoception. The mood regulation compartment deals with self-relevance, prioritization, contingencies and reinforcement. It includes the following areas: medial frontal cortex, medial orbitofrontal cortex, and rostral anterior cingulate cortex. The mood monitoring compartment is involved in evaluating salience and mediating reinforcement, learning, and habit; it contains the ventral striatum-caudate, amygdala, midbrain-ventral tegmental area, and dorsomedial thalamus. Interoception is defined as drive states, autonomic function, and circadian rhythms; it includes the ventral-anterior hippocampus, basal ganglia, anterior insula, hypothalamus, and subcallosal cingulate (SCC). Exteroception is described as attention, appraisal and action; it includes the prefrontal cortex, premotor cortex, parietal cortex, dorsal-posterior hippocampus, mid-cingulate cortex, and posterior cingulate cortex. The regions in each compartment have strong anatomical connections, the reciprocal interaction between compartments provides an insight into treatment response and symptoms presented. The sustained increased activity in ventral limbic (interoceptive) regions and decreased activity in dorsal cortical (exteroceptive) regions corresponds to a negative mood state,

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as seen in a major depressive episode. The reversal of this pattern represents depression remission (Mayberg; 2009). Overall, activity changes within and across compartments directly affect remission. The balance between regions and their sensitivity to specific treatments is still not clear and requires further studies.

An important next step is testing the effect of different treatments, and in this study the focus is repetitive transcranial brain stimulation. The use of fMRI technology can provide critical insights into the circuit patterns of individual subjects to understand the absolute state of activity and how different regions influence each other.

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### METHODS

#### **Patient Population**

Twenty-five currently depressed patients (mean age 42.6, SD = 15.7; 36% male patients) participated in this study after providing informed consent. The Institutional Review Board of Weill Cornell Medical College approved this study and all procedures were conducted accordingly. Patients were recruited from referrals from the outpatient clinic in the Department of Psychiatry at Weill Cornell Medical College and from self-referred patients that contacted the transcranial magnetic stimulation program in this department.

To determine eligibility, all subjects participated in an initial screening interview. To be eligible, patients had to meet the DSM-IV-Text Revision criteria for a major depressive episode and the criteria for treatment resistance. This was defined as failure to respond to at least two previous antidepressant trials at adequate doses for 8 weeks during the current episode. A Board-Certified Psychiatrist determined the diagnosis of the subject in an unstructured clinical interview and through consultation with family members and the current treating psychiatrist. Exclusion criteria included a history of claustrophobia (patients will undergo magnetic resonance imaging (MRI)), seizure disorder or other neurological disorder, head injury resulting in loss of consciousness, metal implants, pacemakers, intrauterine contraceptive devices, or braces, or if they were currently pregnant or lactating. Other exclusion factors were if the subject had bipolar I disorder or a psychotic disorder, was actively suicidal with plan or intent, had been in their current episode for longer than 3 years, had a history of clinically significant personality disorder as

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established in the diagnostic interview, or had substance abuse disorder or substance dependence within the past 3 years.

### **rTMS Treatment Procedure**

The twenty-five patients received daily 10-Hz repetitive transcranial magnetic stimulation (rTMS) over the left dorsolateral prefrontal cortex (dlPFC) five days/week for five weeks. Treatment response was assessed using the 24-item Hamilton Rating Scale for Depression (HAM-D-24) at baseline and one to three days after completion of the rTMS treatment. The rTMS therapy was administered using the NeuroStar TMS Therapy System (Neuronetics, Inc.) with a figure-8 coil with a ferromagnetic core. Each treatment sessions had a total duration of 37.5 min (3000 pulses; 30-second duty cycle, 4 seconds on, 26 seconds off) of 10-Hz excitatory rTMS. The Beam F3 method was used to place the coil and the surface distances between the nasion, inion, tragus and vertex were used as landmarks (Beam, 2009). The resting motor threshold (MT) of each subject was measured before the first rTMS session and on every fifth session. MT was defined as the stimulus strength over the thumb area of motor cortex that produced visually detectable thumb movement on 5 out of 10 consecutive trials. Repetitive stimulation at 120% of the MT was applied.

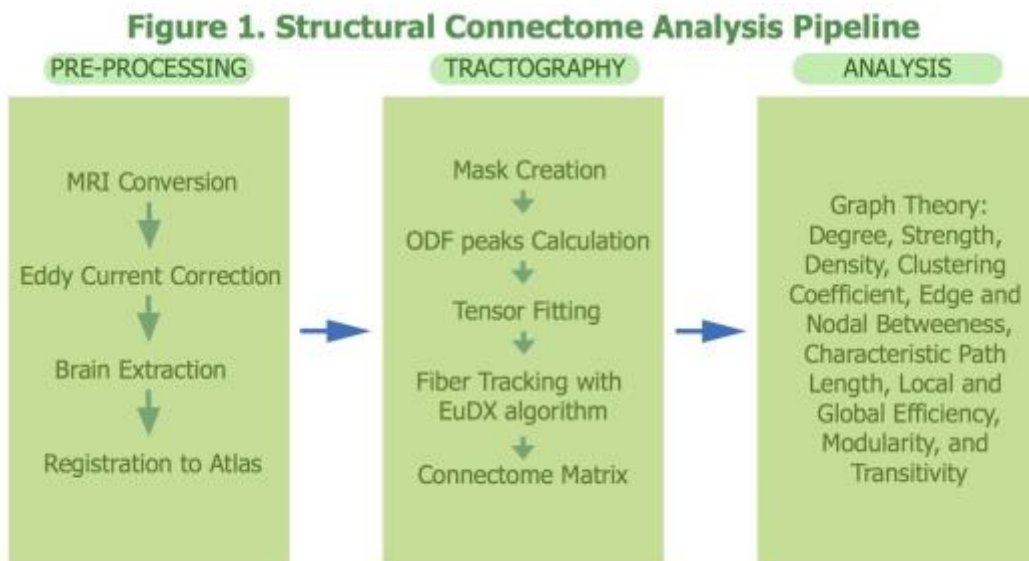
Response to treatment was considered a decrease in depression symptoms as measured by the HAM-D-24 scale. Remission occurs when a patient is asymptomatic, which is defined as no more than minimal symptoms.

### **Neuroimaging Procedure**

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MRIs were acquired within 7 days prior to starting rTMS and within 3 days after the end of the 5-week treatment. Each scanning session was conducted on a 3-T GE scanner (General Electric, Milwaukee, WI) at Weill Cornell and included a high-resolution T1-weighted (MP-RAGE) anatomical scan (256 mm field of view, 256 x 256 voxel acquisition matrix) and diffusion tensor images acquired using a single-shot spin echo imaging sequence. Anatomical scans were acquired in each session for between-session co-registration and for transformation of subjects' imaging data into a common space for group statistics.

### Structural Connectome Analysis



**Figure 1. Structural Connectome Analysis Pipeline.** Graphical representation of the pre-processing and tractography steps to create a structural connectome matrix.

The pre-processing started with the conversion of DICOM images to a NIfTI format using MRICron. The eddy current-induced distortions and subject movements in diffusion data were corrected using the FMRIB's Diffusion Toolbox (FDT) within FSL. The brain was extracted by

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erasing the non-brain tissue from the image of the whole head using the FSL function Brain Extraction Tool (BET). Nodes of the structural brain network were defined as the 96 anatomical brain areas in the Harvard-Oxford atlas. The Harvard-Oxford Atlas (HOA) was coregistered to the subject's native space using FSL's function FLIRT (FMRIB's Linear Image Registration Tool).

Tractography was performed using tools from the dipy python package. The data was fit to a constant solid angle orientation distribution function (ODF) model. An ODF is defined as a distribution of water diffusion as a function of direction. The peaks of ODF are good estimates for the orientation of tract segments at a point in the image. The relative peak threshold was set to 0.5 and the minimum separation angle for tractography to  $25^\circ$ . Diffusion tensors were fit to describe the shape built by the ODF model and all the voxels with a low fractional anisotropy value were rejected. The threshold for fractional anisotropy was low (0.05) because a white matter mask was previously applied. Whole brain deterministic streamline generation was performed using the Euler delta crossings tracking algorithm. Streamlines were generated from eight seeds per voxel. A weighted connectivity matrix was computed based on the number of streamlines traversing between pairwise cortical regions of interest (ROI).

Graph theory metrics were calculated to assess network integration (characteristic path length), distribution (network density), segregation (clustering coefficient, modularity), efficiency (local and global) and nodal influence (nodal degree, betweenness centrality). The characteristic path length is the average shortest path length, the minimum number of edges that must be traversed to go from one node to another, in the network. The network density is the fraction of present connections to possible connections. The clustering coefficient quantifies the number of connections that exist between the nearest neighbors of a node as a proportion of the maximum number of possible connections. Modularity is a statistic that quantifies the degree to which the



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network may be subdivided into clearly delineated groups, which consist of several densely interconnected nodes, and have relatively few connections with nodes in different modules. The global efficiency is the average inverse shortest path length in the network, and is inversely related to the characteristic path length. Random and complex networks have short mean path lengths which means they have high global efficiency of parallel information transfer. Local efficiency is the global efficiency computed on node neighborhoods. The nodal degree is the number of connections that link a node to the rest of the network. The betweenness centrality measure consists of the fraction of all shortest paths in the network that contain a given edge. Edges with high values of betweenness centrality participate in many short paths (Sporns; 2013).

To test for effects of rTMS on structural connectivity, specifically on the brain network characteristics explained above, we used the non-parametric statistical tests: Wilcoxon signed-rank test and the Wilcoxon rank sum test. We used the signed-ranked test to compare each whole-brain network characteristic before and after the rTMS treatment. The rank sum test was used to test the baseline differences of brain network characteristics between responders and non-responders and remitters and non-remitters. The change of brain network characteristics over the course of the treatment was also tested with the groups of responders and non-responders, and remitters and non-remitters. Finally, we did a pairwise comparison between the 96 nodes of the Harvard-Oxford Atlas. We used a multiple linear model to regress measurements in the vectors for the values in the matrices we were comparing to compute the t statistics and p-values of these correlations. The network characteristics (degree, betweenness centrality, clustering coefficient, local efficiency) at baseline were correlated with the baseline Hamilton scale score and the change in Hamilton score for each node in the brain map used. Also, the change in the network characteristic was correlated

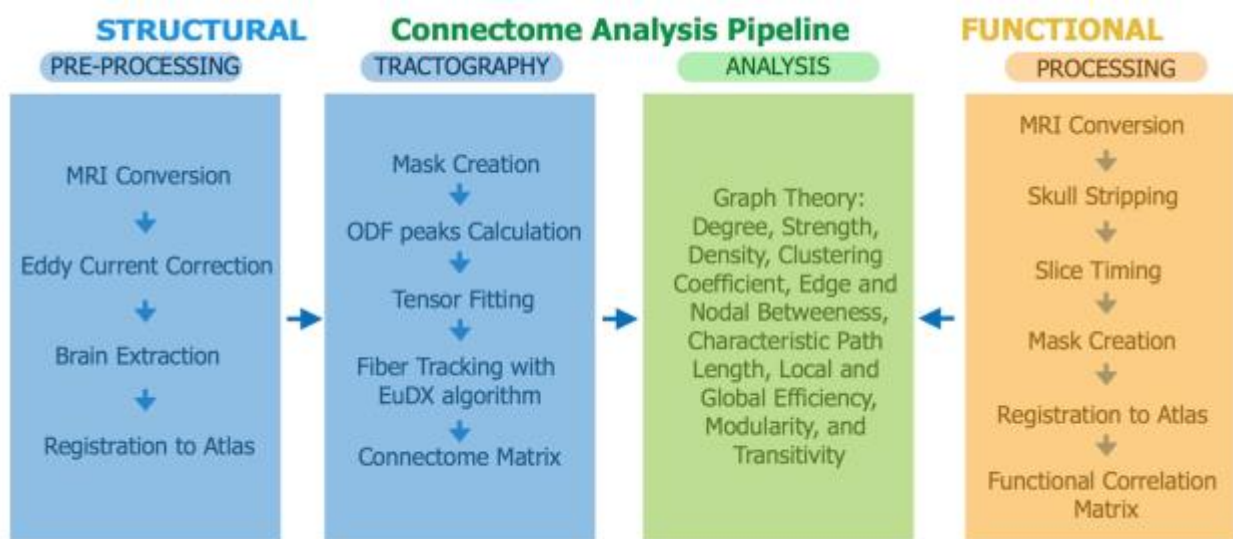
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with the change in Hamilton score. Brain Net was used to visualize the nodes with a significant positive or negative correlation in the comparisons mentioned above.

### *Multiple Iterations*

Iterations are defined as the different runs of the same data through the structural connectome analysis pipeline. Multiple iterations were run for each subject's data before and after the treatment to compare the accuracy of results. Two iterations were compared by subtracting the matrices and creating a histogram, a perfect correlation would have all values centered at zero. For multiple iterations of the same data the matrices were correlated, if the iterations had no outliers then they would have a correlation value of 1 for each pairwise comparison.

### Functional Connectome Analysis



**Figure 2. Structural and Functional Connectome Analysis Pipeline.** Graphical representation of the different steps to build a structural and functional correlation matrix. It includes the analysis performed on both connectome matrices.

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We constructed a node based correlation matrix from resting fMRI raw data. This matrix represents the functional connectivity between different regions, as defined in the Harvard-Oxford brain atlas. We chose this method so we could integrate the results from our structural and functional connectome analyses to perform a whole-brain exploratory study.

The processing of resting fMRI raw data was performed using the python script: FSL resting state pipeline from the Brain Imaging and Analysis Center. Processing was done in four steps: conversion of data to nii format in LAS orientation, skull stripping and average of runs, regression of white matter, and parcellation to produce correlation matrix. First, we changed the orientation of nifti files using BXH tools to LAS, and then converted this data to nii.gz format. LAS orientation is preferred because it is the orientation of the FSL T1 template. We disregarded four time points from the beginning of the run. In the next step, we stripped the skull from the image. Then the functional run was averaged across time using FSL maths. A mask was created using the FSL function Brain Extraction Tool (BET), which was then applied to the entire run of data. In the third step, we took the white matter and cerebrospinal fluid masks generated by FAST and regressed out the signal from the functional data using FSL's mcflirt function. The goal was to fit each subject's data into a template to ensure efficient comparison. In the last step, the Harvard-Oxford Atlas was used as a basis to extract the average time-series for each of the 96 regions. Cross correlation coefficients were found for the entire matrix of 96x96 regions. The output was a matrix with correlation coefficients of average functional activity between regions.

The same graph theory metrics were calculated on the functional connectome matrices, as well as the same non-parametric statistical tests.

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### RESULTS

#### Patient Treatment Results

Twenty-five patients with treatment-resistant depression were enrolled in this study. On average, patient's symptoms improved by 10.16 points on the Hamilton Rating Scale for Depression (HAM-D-24) from baseline to after the treatment (Table 1). Out of the twenty-five patients enrolled nine were considered responders to the treatment (36% response rate). From the patients who responded, 55.6% was the remission rate.

**Table 1**

Patient Characteristics and HAM-D-24 Scores from baseline and post repetitive transcranial magnetic stimulation treatment.

	<b>Baseline (n=25)</b>	<b>Post rTMS (n=25)</b>	<b>p-value</b>
<b>Sex</b>	64% F		
<b>Age</b>	42.60 ± 16.05		
<b>HAM-D</b>	28.08 ± 5.98	17.92 ± 7.92	p < 0.009

#### Structural Connectome Analysis

The structural brain network of all the patients was defined as the 96 anatomical brain areas in the Harvard-Oxford atlas. Relative to baseline, no change was observed post rTMS in the global network metrics of density, characteristic path length, and modularity. No group difference was

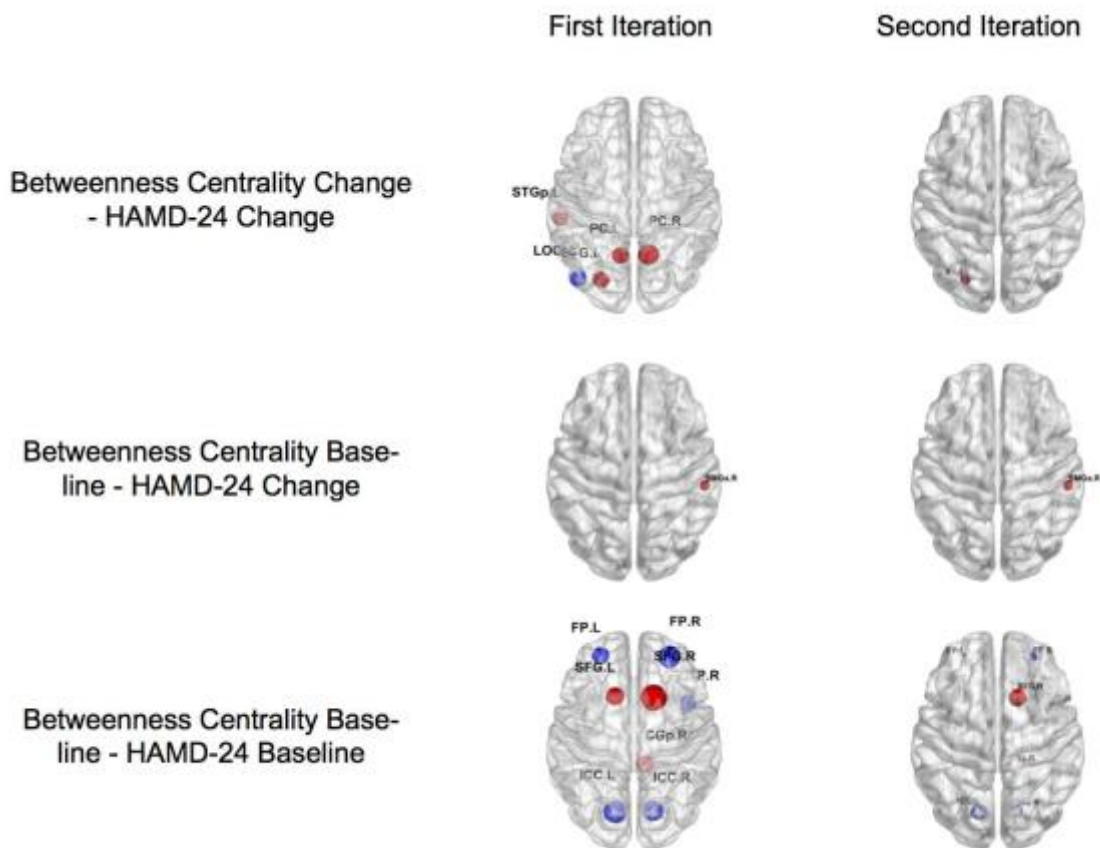
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observed between treatment responders and nonresponders on these global network metrics. Nodal analysis was performed for four graph theory metrics: betweenness centrality, clustering coefficient, degree, and local efficiency. These network properties were correlated with baseline and post treatment change in HAMD-24 score. In the figures for each metric, the node size is proportional to the t-scores (blue: negative correlation, red: positive correlation) in a linear regression with HAMD-24. Two iterations were run for each subject's data before and after the treatment to compare the accuracy of results.

### *Betweenness Centrality*

For the first iteration, the change in betweenness centrality of the left posterior division of the superior temporal gyrus, left precuneus cortex, left occipital fusiform gyrus, and right precuneus cortex were positively correlated with the change in HAMD-24 score before and after the treatment. The left inferior division of the lateral occipital cortex was negatively correlated. In the second iteration, only the left occipital fusiform gyrus was positively correlated with change in HAMD-24 score. For both iterations, betweenness centrality at baseline was positively correlated with change in HAMD-24 score for the right anterior division of the supramarginal gyrus. For both iterations, betweenness centrality at baseline was negatively correlated with HAMD-24 score at baseline for the left frontal pole, left intracalcarine cortex, right intracalcarine cortex, right frontal pole, and right temporal pole. The left superior frontal gyrus and right posterior division of the cingulate gyrus were positively correlated for both iterations. The right superior frontal gyrus was only positively correlated in the first iteration.

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**Figure 3. Correlation between clinical outcome and betweenness centrality for two iterations.**

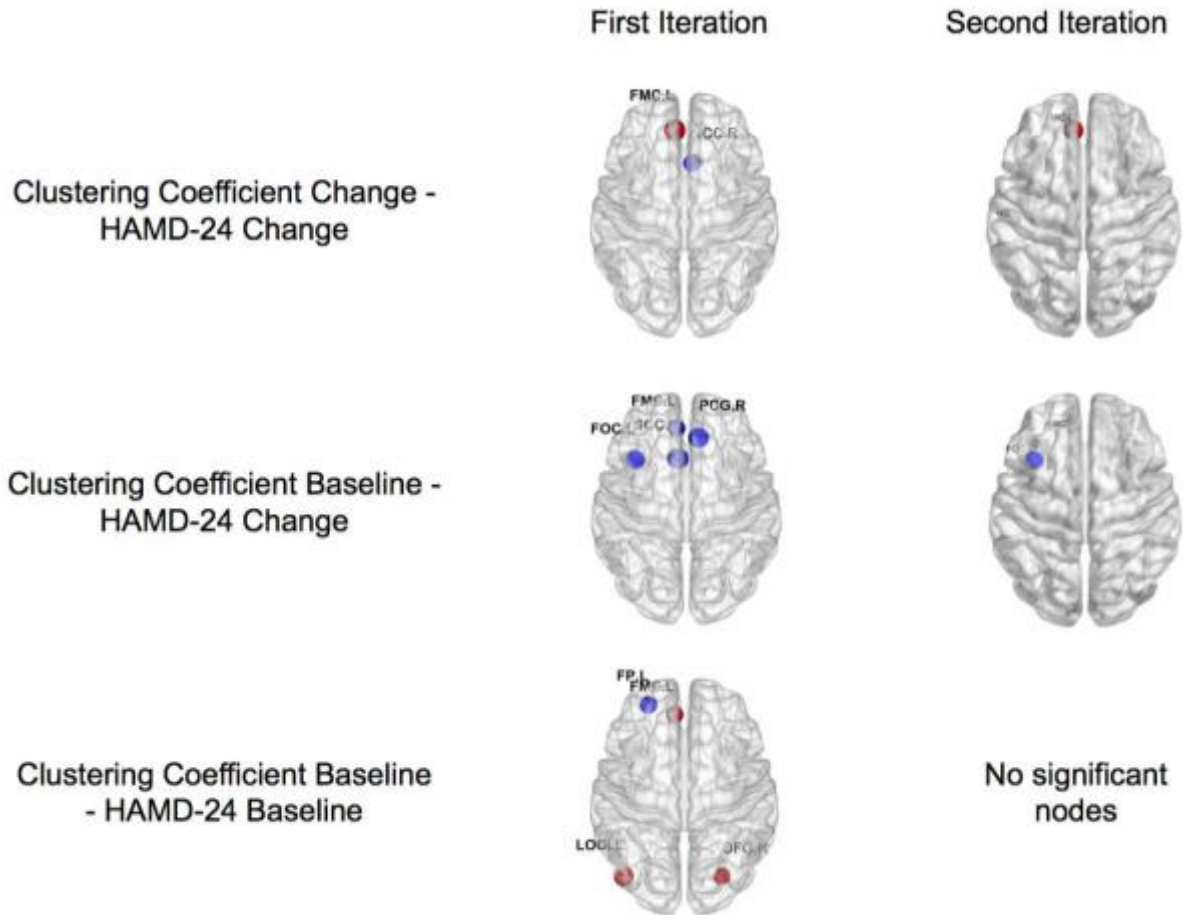
The node size is proportional to the t-scores (blue: negative correlation, red: positive correlation). For the top row, change in betweenness centrality and HAMD-score is correlated in two iterations. First iteration: left posterior division of the superior temporal gyrus (STGp.L), left posterior division of the lateral occipital cortex (LOCp.L), left inferior division of the precuneus cortex (PC.L), left occipital fusiform gyrus (OFG.L), and right precuneus cortex (PC.R); second iteration: the left occipital fusiform gyrus (OFG.L). For the middle row, baseline betweenness centrality is correlated with change in HAMD-24 score in two iterations. Both iterations: left anterior division of the supramarginal gyrus (SMGa.R). In the last row, baseline betweenness centrality is correlated with baseline HAMD-24. First iteration: left frontal pole (FP.L), left superior frontal gyrus (SFG.L), left intracalcarine cortex (ICC.L), right frontal Pole (FP.R), right superior frontal gyrus (SFG.R), right temporal pole (TP.R), right intracalcarine cortex, right posterior division of the cingulate gyrus; second iteration: left frontal pole (FP.L), left intracalcarine cortex (ICC.L), right frontal pole (FP.R), right superior frontal gyrus (SFG.R), right temporal pole (TP.R), right intracalcarine cortex (ICC.R), right posterior division of the cingulate gyrus (CGp.R). Networks are visualized using BrainNet Viewer (<http://www.nitrc.org/projects/bnv/>).

*Clustering Coefficient*

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In both iterations, the change in clustering coefficient for the left frontal medial cortex was positively correlated with the change in HAMD-24 score. The right subcallosal cortex was negatively correlated in the first iteration. The left Heschl's gyrus was positively correlated in the second iteration. For both iterations clustering coefficient at baseline for the left frontal medial cortex and left frontal orbital cortex were negatively correlated with the change in HAMD-24 score. The left subcallosal cortex and right paracingulate gyrus were negatively correlated in the first iteration. The clustering coefficient at baseline for the left inferior division of the lateral occipital cortex, left frontal medial cortex, and right occipital fusiform gyrus were positively correlated with baseline HAMD-24 score. The left frontal pole was negatively correlated in the first iteration. In the second iteration, there were no significant nodes.

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**Figure 4. Correlation between clinical outcome and clustering coefficient for two iterations.** The node size is proportional to the t-scores (blue: negative correlation, red: positive correlation). For the top row, change in clustering coefficient and HAMD-score is correlated in two iterations. First iteration: left frontal medial cortex (FMC.L), right subcallosal cortex (SCC.R); second iteration: left frontal medial cortex (FMC.L), left heschl's gyrus (HG.L). For the middle row, baseline clustering coefficient is correlated with change in HAMD-24 score in two iterations. First iteration: left frontal medial cortex (FMC.L), left subcallosal cortex (SCC.L), left frontal orbital cortex (FOC.L), right paracingulate gyrus (PCG.R); second iteration: left frontal medial cortex (FMC.L), left frontal orbital cortex (FOC.L). In the last row, baseline clustering coefficient is correlated with baseline HAMD-24. First iteration: left frontal pole (FP.L), left inferior division of the lateral occipital cortex (LOCi.L), left frontal medial cortex (FMC.L), right occipital fusiform gyrus (OFG.R); second iteration: no significant nodes. Networks are visualized using BrainNet Viewer (<http://www.nitrc.org/projects/bnv/>).

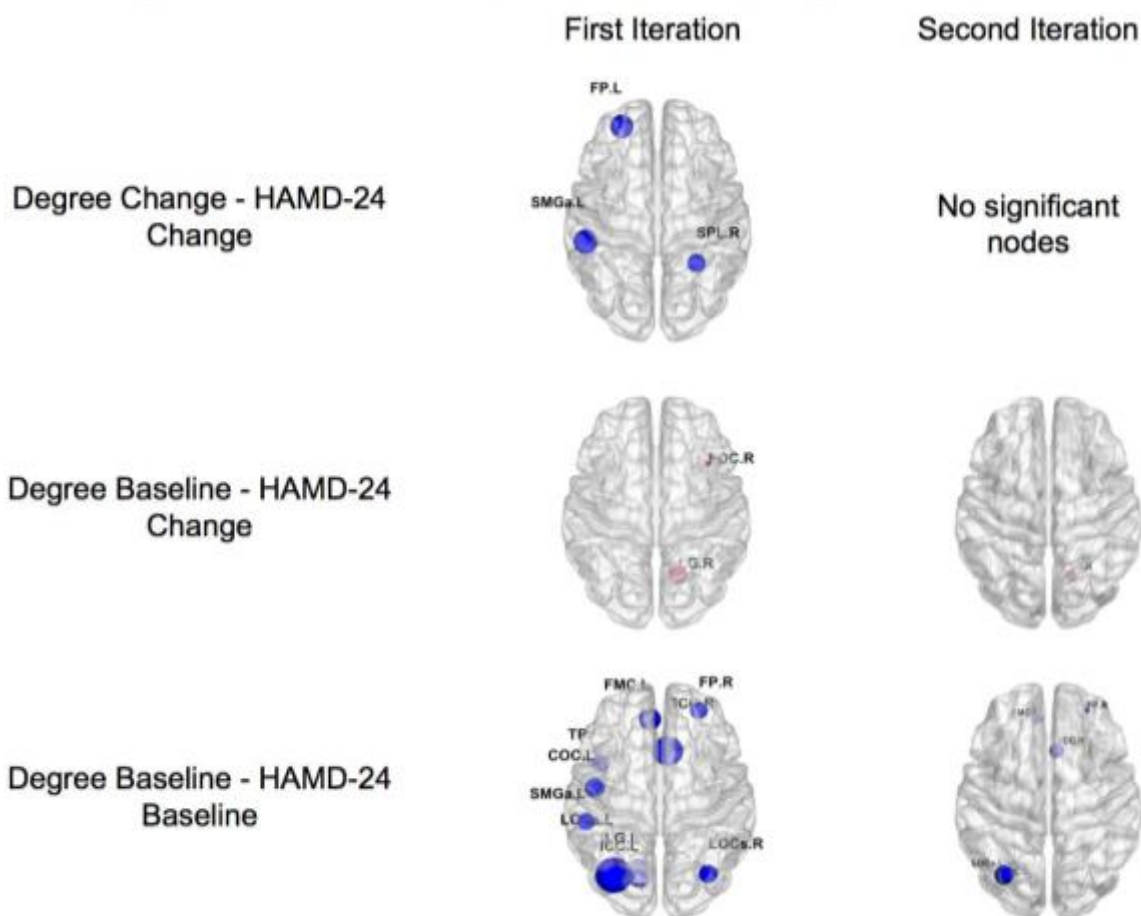
### *Degree*

For the first iteration, the change in degree for the left frontal pole, left anterior division of the supramarginal gyrus, and right superior parietal lobule were negatively correlated with the



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change in HAMD-24 score. In the second iteration, there were no significant nodes. For both iterations, degree at baseline for the right lingual gyrus was positively correlated with HAMD-24 change. The right frontal orbital cortex was positively correlated for the first iteration. For both iterations, degree at baseline for the left superior division of the lateral occipital cortex, left intracalcarine cortex, left frontal medial cortex, right frontal pole, and right subcallosal cortex were negatively correlated with baseline HAMD-24 score. In the first iteration, the left temporal pole, left anterior division of the supramarginal gyrus, left lingual gyrus, left central opercular cortex, and right superior division of the lateral occipital cortex were negatively correlated.



**Figure 5. Correlation between clinical outcome and degree for two iterations.** The node size is proportional to the t-scores (blue: negative correlation, red: positive correlation). For the top row, change in degree and HAMD-score is correlated in two iterations. First iteration: left frontal

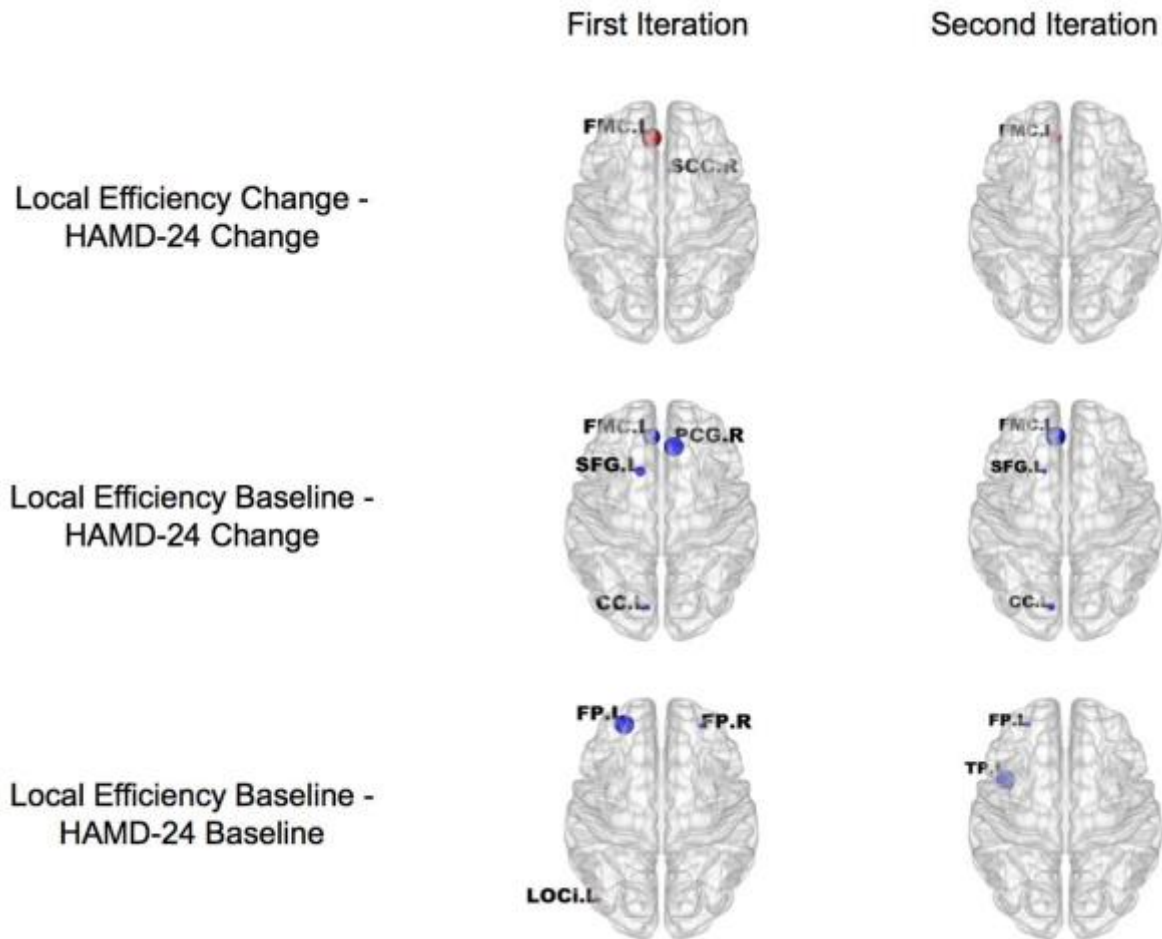
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pole (FP.L), left anterior division of the supramarginal gyrus (SMGa.L), right superior parietal lobule (SPL.R); second iteration: no significant nodes. For the middle row, baseline degree is correlated with change in HAMD-24 score in two iterations. First iteration: right lingual gyrus (LG.R), right frontal orbital cortex (FOC.R); second iteration: right lingual gyrus (LG.R). In the last row, baseline degree is correlated with baseline HAMD-24. First iteration: left temporal pole (TP.L), left anterior division of the supramarginal gyrus (SMGa.L), left superior division of the lateral occipital cortex (LOCs.L), left Intracalcarine cortex (ICC.L), left frontal medial cortex (FMC.L), left lingual gyrus (LG.L), left central opercular cortex (COC.L), right frontal pole (FP.R), right superior division of the lateral occipital cortex (LOCs.R), right subcallosal cortex (SCC.R); second iteration: left superior division of the lateral occipital cortex (LOCs.L), left intracalcarine cortex (ICC.L), left frontal medial cortex (FMC.L), right frontal pole (FP.R), right subcallosal cortex (SCC.R). Networks are visualized using BrainNet Viewer (<http://www.nitrc.org/projects/bnv/>).

### *Local Efficiency*

For both iterations, the change in local efficiency for the left frontal medial cortex was positively correlated with change in HAMD-24 score. The right subcallosal cortex was negatively correlated for the first iteration. For both iterations, local efficiency at baseline for the left superior frontal gyrus, left frontal medial cortex, and left cuneal cortex were negatively correlated with the change in HAMD-24 score. The right paracingulate gyrus was negatively correlated in the first iteration. In both iterations the local efficiency at baseline of the left frontal pole was negatively correlated with the baseline HAMD-24 score. The left inferior division of the lateral occipital cortex was positively correlated and the right frontal pole was negatively correlated in the first iteration. The left temporal pole was negatively correlated in the second iteration.

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**Figure 6. Correlation between clinical outcome and local efficiency for two iterations.** The node size is proportional to the t-scores (blue: negative correlation, red: positive correlation). For the top row, change in local efficiency and HAMD-score is correlated in two iterations. First iteration: left frontal medial cortex (FMC.L), right subcallosal cortex (SCC.R); second iteration: left frontal medial cortex (FMC.L). For the middle row, baseline local efficiency is correlated with change in HAMD-24 score in two iterations. First iteration: left superior frontal gyrus (SFG.L), left frontal medial cortex (FMC.L), left cuneal cortex (CC.L), right paracingulate gyrus (PCG.R); second iteration: left superior frontal gyrus (SFG.L), left frontal medial cortex (FMC.L), left cuneal cortex (CC.L). In the last row, baseline local efficiency is correlated with baseline HAMD-24. First iteration: left frontal pole (FP.L), left inferior division of the lateral occipital cortex (LOCi.L), right frontal pole (FP.R); second iteration: left frontal pole (FP.L), left temporal pole (TP.L). Networks are visualized using BrainNet Viewer (<http://www.nitrc.org/projects/bnv/>).

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### *Multiple Iterations*

To understand the discrepancies between iterations, like some significant nodes not showing in iterations of the same data, multiple iterations of the same data were run through the structural connectome analysis pipeline, seen in Figure 1, and compared. Histograms were created for the difference of two matrices from different iterations of the same data. This was done for the pre and post treatment data of twenty-five patients, generating fifty histograms. All histograms had most values at zero, as expected for a perfect match between matrices of two iterations. The streamline count from one iteration to another of the same data can be very different in some cells of the matrix, these outliers increase the range of possible values in this difference matrix.

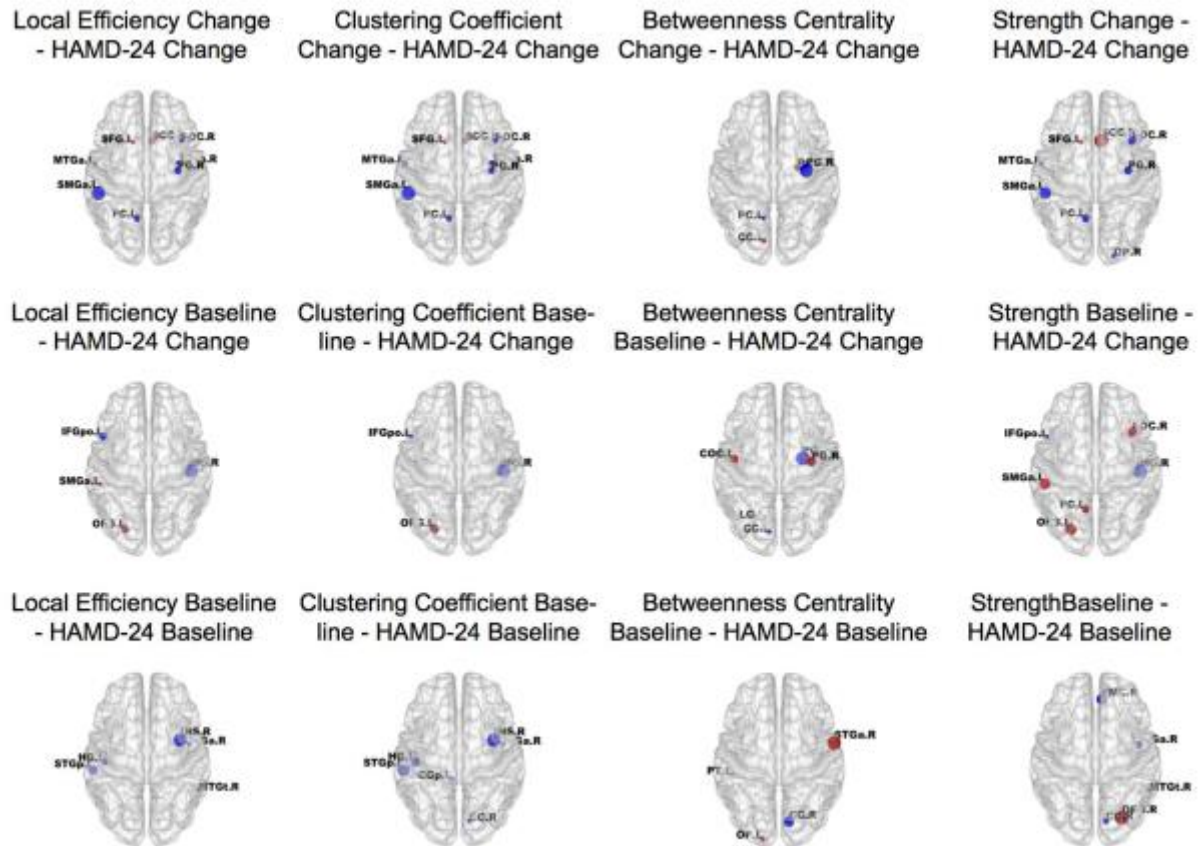
To further analyze the differences between iterations, data from nine subjects was run for 5-10 iterations. The structural connectome matrices from the different iterations of the same data were correlated. The majority (91.78%) of iterations had a correlation value of one, a perfect correlation.

### **Functional Connectome Analysis**

The functional brain network of all the patients was defined as the 96 anatomical brain areas in the Harvard-Oxford atlas. Nodal analysis was performed for four graph theory metrics: betweenness centrality, clustering coefficient, strength, and local efficiency. We used strength instead of degree because the functional connectivity matrices are not binary, thus degree does not compute the number of links connected to a given node accurately. Strength is the sum of weights of links connected to the node, thus measuring the same property as degree. These network properties were correlated with baseline and post treatment change in HAMD-24 score. In the

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figures for each metric, the node size is proportional to the t-scores (blue: negative correlation, red: positive correlation) in a linear regression with HAMD-24.



**Figure 7. Functional Connectome Analysis: Correlation between graph theory metrics and clinical outcome for a brain map parcellation of 96 regions of interest.** The node size is proportional to the t-scores (blue: negative correlation, red: positive correlation). For the top row, change in network metric was correlated with change in HAMD-24 score. Local efficiency: left superior frontal gyrus (SFG.L), left anterior division of the middle temporal gyrus (MTGa.L), left anterior division of the supramarginal gyrus (SMGa.L), left precuneus cortex (PC.L), right precentral gyrus (PG.R), right subcallosal cortex (SCC.R), right anterior division of the temporal fusiform cortex (TFCa.R), right frontal occipital cortex (FOC.R); clustering coefficient: left superior frontal gyrus (SFG.L), left anterior division of the middle temporal gyrus (MTGa.L), left anterior division of the supramarginal gyrus (SMGa.L), left precuneus cortex (PC.L), right precentral gyrus (PG.R), right subcallosal cortex (SCC.R), right anterior division of the temporal fusiform cortex (TFCa.R), right frontal occipital cortex (FOC.R); betweenness centrality: left precuneus cortex (PC.L), left cuneal cortex (CC.L), right precentral gyrus (PG.R), right anterior division of the parahippocampal gyrus (PGa.R); strength: left superior frontal gyrus (SFG.L), left anterior division of the middle temporal gyrus (MTGa.L), left anterior division of the

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supramarginal gyrus (SMGa.L), left precuneus cortex (PC.L), right precentral gyrus (PG.R), right subcallosal cortex (SCC.R), right frontal orbital cortex (FOC.R), right occipital pole (OP.R). For the middle row, the baseline measure of the network metric was correlated with change in HAMD-24 score. Local efficiency: left pars opercularis of the inferior frontal gyrus (IFGpo.L), left anterior division of the supramarginal gyrus (SMGa.L), left occipital fusiform gyrus (OFG.L), right heschl's gyrus (HG.R); clustering coefficient: left pars opercularis of the inferior frontal gyrus (IFGpo.L), left occipital fusiform gyrus (OFG.L), right heschl's gyrus (HG.R); betweenness centrality: left cuneal cortex (CC.L), left lingual gyrus (LG.L), left central opercular cortex (COC.L), right precentral gyrus (PG.R), right anterior division of the parahippocampal gyrus (PGa.R); strength: left pars opercularis of the inferior frontal gyrus (IFGpo.L), left anterior division of the supramarginal gyrus (SMGa.L), left precuneus cortex (PC.L), left occipital fusiform gyrus (OFG.L), right frontal orbital cortex (FOC.R), right heschl's gyrus (HG.R). For the bottom row, the baseline measure of the network metric was correlated with baseline HAMD-24 score. Local efficiency: left posterior division of the superior temporal gyrus (STGp.L), left heschl's gyrus (HG.L), right insular cortex (INS.R), right temporo-occipital part of the middle temporal gyrus (MTGt.R), and right anterior division of the inferior temporal gyrus (ITGa.R); clustering coefficient: left posterior division of the superior temporal gyrus (STGp.L), left posterior division of the cingulate gyrus (CGp.L), left Heschl's Gyrus (HG.L), right insular cortex (INS.R), right anterior division of the inferior temporal gyrus (ITGa.R), and right cuneal cortex (CC.R); betweenness centrality: left planum temporale (PT.L), left occipital pole (OP.L), right anterior division of the superior temporal gyrus (STGa.R), right cuneal cortex (CC.R); strength: right temporo-occipital part of the middle temporal gyrus (MTGt.R), and right anterior division of the inferior temporal gyrus (ITGa.R), right cuneal cortex (CC.R), right occipital fusiform gyrus (OFG.L). Networks are visualized using BrainNet Viewer (<http://www.nitrc.org/projects/bnv/>).

### *Betweenness Centrality*

The change in betweenness centrality of the left precuneus cortex and right precentral gyrus were negatively correlated with the change in HAMD-24 score before and after the treatment. The left cuneal cortex and right anterior division of the parahippocampal gyrus were positively correlated. Betweenness centrality at baseline for the left cuneal cortex, left lingual gyrus, and right anterior division of the parahippocampal gyrus were negatively correlated with change in HAMD-24 score. The left central opercular cortex and right precentral gyrus were positively correlated. Betweenness centrality at baseline for the left planum temporale, left occipital pole, and right anterior division of the superior temporal gyrus were positively correlated with HAMD-24 score at baseline. The right cuneal cortex was negatively correlated.

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### *Clustering Coefficient*

The change in clustering coefficient of the right anterior division of the temporal fusiform cortex, right frontal orbital cortex, left anterior division of the middle temporal gyrus, left anterior division of the supramarginal gyrus, left precuneus cortex and right precentral gyrus were negatively correlated with the change in HAMD-24 score before and after the treatment. The left superior frontal gyrus and right subcallosal cortex were positively correlated. Clustering coefficient at baseline was negatively correlated with change in HAMD-24 score for the left pars opercularis of the inferior frontal gyrus and right Heschl's Gyrus. The left occipital fusiform gyrus was positively correlated. Clustering coefficient at baseline was negatively correlated with HAMD-24 score at baseline for the left posterior division of the superior temporal gyrus, left posterior division of the cingulate gyrus, left Heschl's Gyrus, right insular cortex, right anterior division of the inferior temporal gyrus, and right cuneal cortex.

### *Strength*

The change in strength of the left anterior division of the middle temporal gyrus, left anterior division of the supramarginal gyrus, left precuneus cortex, right precentral gyrus, right frontal orbital cortex, and right occipital pole were negatively correlated with the change in HAMD-24 score before and after the treatment. The left superior frontal gyrus and right subcallosal cortex were positively correlated. Strength at baseline was positively correlated with change in HAMD-24 score for the left anterior division of the supramarginal gyrus, left precuneus cortex, left occipital fusiform gyrus, and right frontal orbital cortex. The left pars opercularis of the inferior frontal gyrus and right Heschl's Gyrus were negatively correlated. Strength at baseline

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was negatively correlated with HAMD-24 score at baseline for the right temporo-occipital part of the middle temporal gyrus, right anterior division of the inferior temporal gyrus, right frontal medial cortex, and right cuneal cortex. The right occipital fusiform gyrus was positively correlated.

### *Local Efficiency*

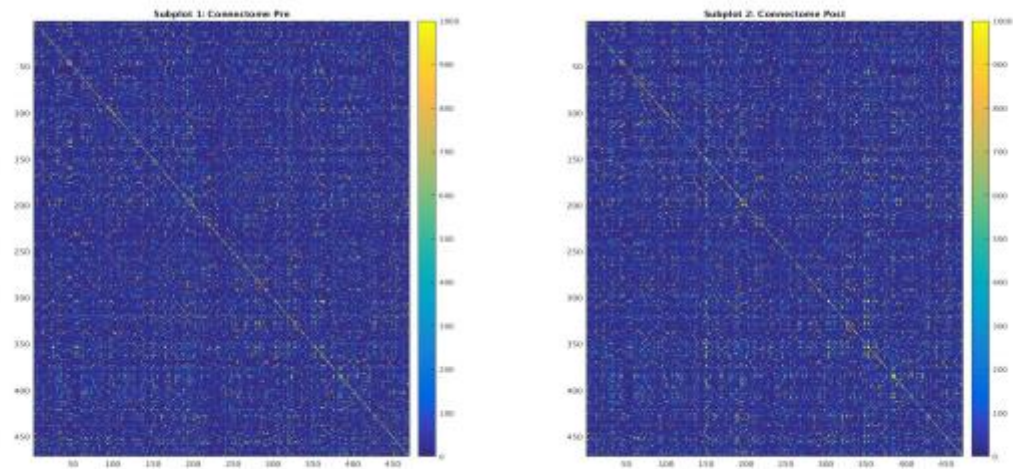
The change in local efficiency of the left anterior division of the middle temporal gyrus, left anterior division of the supramarginal gyrus, left precuneus cortex, right precentral gyrus, right anterior division of the temporal fusiform cortex, and right frontal orbital cortex were negatively correlated with the change in HAMD-24 score before and after the treatment. The right subcallosal cortex and left superior frontal gyrus were positively correlated. Local efficiency at baseline was positively correlated with change in HAMD-24 score for the left anterior division of the supramarginal gyrus and left occipital fusiform gyrus. The left pars opercularis of the inferior frontal gyrus and right Heschl's gyrus were negatively correlated. Local efficiency at baseline was negatively correlated with HAMD-24 score at baseline for the left posterior division of the superior temporal gyrus, left Heschl's gyrus, right insular cortex, right temporo-occipital part of the middle temporal gyrus, and right anterior division of the inferior temporal gyrus.

### **Exploratory Section**

The structural brain network of the twenty-five patients was defined as the 471 anatomical brain areas in the Harvard-Oxford atlas, including cortical and subcortical regions.



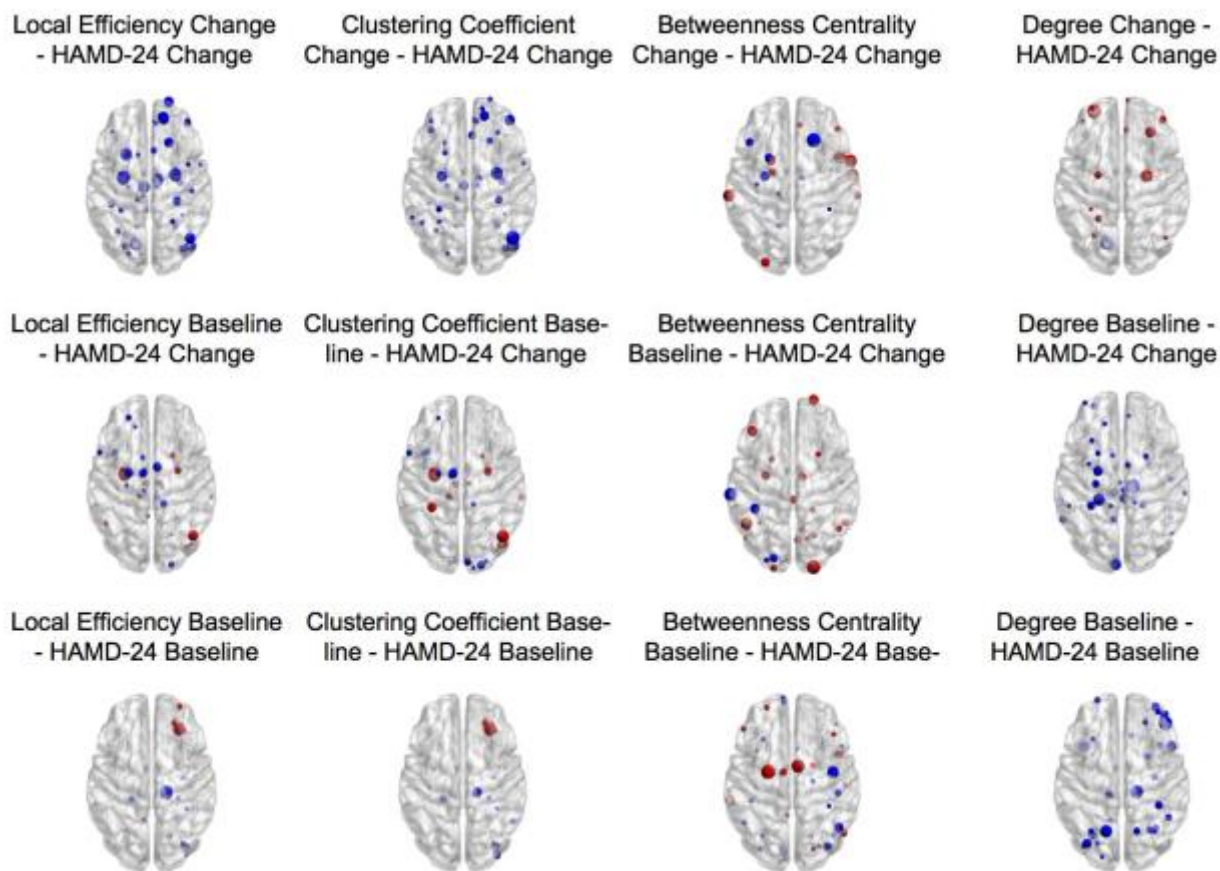
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**Figure 8. Visualization of the structural connectome matrices before and after treatment for the first subject.** The structural brain network of this patient was parcellated per the Harvard-Oxford Atlas with 471 regions of interest. The color bar on the right of the correlation matrices represents the strength of the correlation, yellow being the strongest. Each dot on the matrix is the correlation between two regions of the 471x471 matrix.

Nodal analysis was performed for four graph theory metrics: betweenness centrality, clustering coefficient, degree, and local efficiency. The baseline results were correlated with the baseline HAMD-24 score and the change in HAMD-24 score for each node. The change in network characteristic was correlated with the change in HAMD-24 score.

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**Figure 9. Correlation between graph theory metrics and clinical outcome of a network with 471 regions of interest.** The brain atlas used was the Harvard-Oxford Atlas with 471 regions of interest, which includes cortical and subcortical divisions. The node size is proportional to the t-scores (blue: negative correlation, red: positive correlation). Networks are visualized using BrainNet Viewer (<http://www.nitrc.org/projects/bnv/>).

### DISCUSSION

#### **Effect of rTMS on Structural Brain Connectivity**

##### *Biomarker for Depression Severity*

The baseline correlation between network characteristic and HAMD-24 score provides potential biomarkers for depression severity. The frontal pole has baseline betweenness centrality, clustering coefficient, degree, and local efficiency negatively correlated with baseline HAMD-24; this could be a potential biomarker to predict depression severity. The frontal pole has been associated with the midline core in the default mode network (DMN) and the exteroception compartment of the depression network (DN). Decreased activity in dorsal cortical (exteroceptive) regions corresponds to a negative mood state, this relationship requires further study considering this region's connections with other parts of the brain.

The temporal pole has baseline betweenness centrality and degree negatively correlated with HAMD-24. This area is part of the dorsal medial prefrontal cortex (dmPFC) subsystem in the DMN, which is mainly active when considering the present. The left frontal medial cortex has baseline degree negatively correlated and clustering coefficient positively correlated with HAMD-24 score, this region is part of the mood regulation compartment in the DN. Both metrics quantify the connections that link the node to the network, the clustering coefficient focuses on the connections from nearest neighbors and degree on the entire network. A potential area of study is how this region's connectivity with the rest of the network predicts depression severity. The right subcallosal cortex has baseline degree negatively correlated with HAMD-24 score, and it is part of the interoception compartment in the DN. The superior frontal gyrus has baseline betweenness

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centrality that is positively correlated with HAMD-24 score, this region is included in the dMPFC subsystem of the DMN and the mood regulation compartment of the DN. The right posterior division of the cingulate gyrus has baseline betweenness centrality that is also positively correlated, it is one of the “hubs” in the midline core of the DMN, a key area for study. The left frontal medial cortex has baseline clustering coefficient positively correlated with HAMD-24 score, and it is part of the mood regulation compartment of the DN.

The intracalcarine cortex, located within the supracalcarine cortex of the occipital lobe, has baseline betweenness centrality and degree negatively correlated with baseline HAMD-24 score. The left inferior division of the lateral occipital cortex has baseline local efficiency and clustering coefficient positively correlated with HAMD-24 score. These two areas are not identified in the DMN or DN suggesting further studies are necessary to understand their role as potential biomarkers for depression severity.

### *Biomarker for Treatment Response*

The correlation between baseline network metric and the change in HAMD-24 score can provide us with potential biomarkers that predict response to rTMS treatment.

The left frontal medial cortex and right paracingulate gyrus have baseline clustering coefficient and local efficiency that is negatively correlated with change in HAMD-24. They are both part of the mood regulation compartment of the DN. The left frontal orbital cortex has baseline clustering coefficient negatively correlated and the right frontal orbital cortex has baseline degree positively correlated with change in HAMD-24 score. This region is part of the mood regulation compartment of the DN. The bilateral difference is a potential area for study to understand how it can predict response to treatment. The left subcallosal cortex has baseline clustering coefficient that is

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negatively correlated with change in HAMD-24, this region is part of the interoceptive compartment of the DN. The left superior frontal gyrus has baseline local efficiency that is negatively correlated with change in HAMD-24, it is part of the dMPFC subsystem of the DMN and the mood regulation compartment of the DN.

### *Treatment Effect on Structural Connectivity*

The correlation between the change in network metric and HAMD-24 score throughout the treatment can help us understand the pathophysiology of depression and how this treatment affects brain networks. The post treatment change in clustering coefficient and local efficiency in the right subcallosal cortex was negatively correlated with change in HAMD-24; this region is part of the interoception compartment in the DN. The post treatment change in degree of the right superior parietal lobule was negatively correlated with the change in HAMD-24, this region is part of the interoceptive compartment in the DN. The sustained increased activity in ventral limbic (interoceptive) regions corresponds to a negative mood state; thus, further studies on these regions could help us understand how rTMS affects mood. The post treatment change in the left frontal pole is negatively correlated with the change in HAMD-24, this region has been associated with the midline core in the default mode network (DMN) and the exteroception compartment of the depression network (DN). This region was also identified as a potential biomarker for depression severity, further studies on how it changes over the course of treatment can help us understand the role of the frontal pole in depression severity and treatment response. The post treatment change in local efficiency and clustering coefficient of the left frontal medial cortex is positively correlated with the change in HAMD-24. This region is part of the mood regulation compartment in the DN. The post treatment change in betweenness centrality in the left posterior division of the superior

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temporal gyrus and precuneus cortex were positively correlated with change in HAMD-24. The superior temporal gyrus is part of the MTL subsystem of the DMN and the precuneus cortex is part of the exteroception compartment of the DN.

### *Finer Parcellation*

The brain map was registered to a Harvard-Oxford Atlas with 471 anatomical brain areas and run through our structural connectome analysis pipeline. This determined it is feasible to conduct a connectome analysis with a higher number of regions of interest, which allows us to have a finer division of brain regions. This can further our knowledge of the pathophysiology of depression and the effect of TMS in the brain, allowing us to target brain regions with TMS more accurately. A limitation in this approach is that a bigger sample size is necessary because multiple comparisons between more regions of interest leads to less statistical power.

## **Effect of rTMS on the Structural and Functional Connectivity**

### *Biomarker for Treatment Response*

Strength at baseline was positively correlated with change in HAMD-24 score for the right frontal orbital cortex. This region is part of the mood regulation compartment of the DN. A potential area of study is the number of anatomical and functional connections this region has with the entire cortex and how this can predict whether the person will respond to treatment.

### *Treatment Effect on Structural and Functional Connectivity*

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The change in strength of the left anterior division of the supramarginal gyrus was negatively correlated with the change in HAMD-24 score before and after the treatment. This region is part of the exteroception compartment of the DN, where decreased activity is correlated with negative mood. Thus, a study on the change of anatomical and functional connections this region has with the entire cortex over the course of treatment can provide an insight into how rTMS affects mood.

In functional connectivity, the change in clustering coefficient and local efficiency of the right subcallosal cortex was positively correlated with the change in HAMD-24 score throughout the treatment. In structural connectivity, it was negatively correlated. An improvement in depression symptoms, decreased HAMD-24, would mean a decrease in functional connections but an increase in the anatomical connections between this region and its nearest neighbors. Understanding this would help explain the role of the subcallosal cortex in the interoception compartment of the DN.

For functional connectivity, the change in betweenness centrality of the left precuneus cortex was negatively correlated with the change in HAMD-24 score before and after the treatment. In structural connectivity, it was positively correlated. This region is part of the exteroception compartment of the DN. The opposite correlations for structural and functional data requires further study.

### **Limitations**

Each cell in the structural connectome matrix represents the streamline count between two regions. We found variability in the streamline count of some regions between iterations of the

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same data. A possible source of error could be in the seeding step of tractography, because seeding is performed in a random manner within a voxel of the brain map. This means that tracking starts at different positions within the voxel for each iteration. Efforts to minimize this error can be running multiple iterations of the same data and taking an average connectome matrix of all the iterations.

For the resting fMRI processing we did not include all the pre-processing steps, like motion correction, because it did not significantly alter the results and it made the process faster. Another potential limitation was the lower image quality from registering the functional data on the MNI template. Instead, we could register it to a template derived from our structural data for each subject so we have a better image to analyze. This would improve localization of the effects of rTMS on the functional-structural connectivity.

A limitation for all the results described in this study is the lack of a control group. Future studies should include a control group of healthy subjects and subjects with major depressive disorder that did not receive rTMS treatment. This is necessary to verify the potential biomarkers described for depression and other results, ensuring they are not due to an error in the treatment or analysis protocol.

We don't have enough statistical power to prove the hypothesis drawn from this study due to the small sample size. However, this is an exploratory analysis that aims to articulate hypotheses about the most significant regions that should be the focus of future studies.



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### **Future Directions**

Various regions were identified as potential areas for future study. Most notably, the frontal pole was described as a potential biomarker for depression severity and could also provide information on the pathophysiology of depression and the effect of rTMS. The frontal pole, Brodmann Area 10, could be a potential target for neuromodulation with rTMS. Areas that are not included in the default mode network or depression network such as: intracalcarine cortex and lateral occipital cortex were identified as potential biomarkers for treatment response. These offer the possibility of designing a prospective study with an a priori hypothesis using TMS, or different brain stimulation modalities, to affect these brain regions that can predict treatment response or depression severity. These results are based on a restricted set of brain stimulation parameters, specifically the left dlPFC, so future studies that target the significant nodes identified in this study could further our knowledge on depression pathophysiology.

To explore the relationship between structural and functional connectivity, future studies should focus on the subcallosal cortex, orbitofrontal cortex, and supramarginal gyrus. These specific regions were identified as potential biomarkers for treatment response and their change across the treatment protocol could explain the simultaneous effect of rTMS on structural and functional connectivity. This could help us understand how functional outcome is constrained by the structural connectivity and how to integrate analytic tools to explore this relationship. Electric field modelling can be used to target brain regions that are potential biomarkers for treatment response or depression severity. Ultimately creating a method that has structural-functional analytical tools to identify regions of interest, and electric field modelling to ensure the TMS coil is targeting these areas accurately.

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### CONCLUSION

This was a clinical trial that used the FDA-approved rTMS protocol to treat patients with major depressive disorder. Part of the story of treating depression is understanding how the brain is changed with TMS, which is why we decided to explore structural and functional connectivity. Our aim was to find biomarkers for depression severity and treatment response. Using a structural and functional connectome analytic pipeline we identified various regions, as described above, that are consistent with what we think about brain networks, namely the default mode network and circuit model of depression. This style to analyze whole-brain structural and functional connectivity and graph theory techniques to characterize brain architecture can be useful to develop objective measures of brain connectivity related to depression severity, treatment outcome and depression pathophysiology.

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